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Study on the Mechanism of Pollen Typhae in the Treatment of Schizophrenia Based on Network Pharmacology

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Abstract:

To explore the lipid-lowering mechanism of walnut meal based on network pharmacology. The active components and corresponding potential targets of Pollen Typhae were obtained through TCMSP database and literature mining; Use genecards, OMIM and other databases to predict and screen schizophrenia related genes; Cytoscape 3.7.2 software was used to construct the "drug active ingredient disease target" network to form the component and target interaction relationship of Pollen Typhae in the treatment of schizophrenia, and go function and KEGG pathway enrichment analysis were carried out. Eight active components of walnut meal were screened, six of which had 135 schizophrenia related targets, AKT1, IL6 and IL1-β, CASP3, VEGFA, JUN and PTGS2 may be the key targets of schizophrenia. The main signal pathways are lipid and atherosclerosis, fluid shear stress and atherosclerosis, chemical carcinogenesis - receptor activation, AGE-RAGE signaling pathway in diabetic complexes, human cytomegalovirus infection, TNF signaling pathway Chemical carcinogenesis - reactive oxygen species. Pollen Typhae can treat schizophrenia through multi-component, multi-target and multi-channel. The most targeted components are kaempferol, luteolin and quercetin; The most affected targets were PPARG, PTGS1, PTGS2, NCOA2 and CASP3.

Keywords*: Pollen Typhae, Schizophrenia, TCMSP, OMIM.*

I. INTRODUCTION

Schizophrenia is a serious mental disease characterized by many obstacles such as cognition, emotion, will and behavior and the disharmony between mental activities and the environment, accompanied by a serious decline in social function [1,2]. With the rapid development of China's economy and the

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transformation stage of society, people's lives and work pressure have led to the increasing incidence rate of mental illness. Schizophrenia has become one of the most serious diseases in the world. At present, the pathogenesis of schizophrenia is still unclear, and the development of related drugs is only aimed at the improvement of some symptoms. The research and development of drugs needs further development [3].

Pollen Typhae is the dried pollen of Typha angustifolial L., Typha Orientalis Presl or similar plants in the Typhaceae. The medicinal records of Puhuang began in Shennong herbal classic, which is listed as the top grade and has the effect of promoting blood circulation and removing blood stasis; It was first recorded in rihuazi materia medica that its processed products had hemostatic effect after processing [4]. The effective components in Typha are flavonoids, such as naringin, quercetin, Typha neoglycoside, etc. it also contains hemostatic tannin, steroids, alkanes and sugars [5,6]. In clinic, fried pollen Typhae and charcoal pollen Typhae are mostly used for various hemorrhagic disease syndromes such as metrorrhagia and leakage. Pollen typhus is used to treat chest and abdominal pain and amenorrhea caused by blood stasis. Modern clinical studies have shown that pollen cattail can also treat coronary heart disease, atherosclerosis, diabetes and hyperlipidemia. At the same time, pollen is used as a compatibility medicine for schizophrenia, but its specific mechanism is not clear. It is necessary to further study [7,8]. Based on this, this study analyzed the functional components, targets and mechanism of Pollen Typhae in the treatment of schizophrenia through network pharmacological methods, so as to provide more scientific basis for Pollen Typhae in the treatment of schizophrenia.

II. MATERIALS AND METHODS

2.1 Screening of active Components and Potential Targets of Pollen Typhae

By retrieving TCMSP(http://lsp.nwu.edu.cn/tcmsp.php)The main effective components of Pollen Typhae were obtained from the online database of pharmacology [9], and the main effective components of Pollen Typhae were screened under the conditions of oral bioavailability (OB)≥30% and drug likeness (DL)≥0.18 [10,11]. The "target information" function in TCMSP was used to screen the drug targets of effective active ingredients.

2.2 Disease Target Collection

Using the genome annotation (Genecards) database(https://www.genecards.org/), input the keyword "schizophrenia" into the human gene database and export the target genes related to schizophrenia in Excel format; Enter the human Mendelian inheritance (OMIM) database(https://omim.org/), enter the "genemaps" function, input the keyword "schizophrenia", and export the target genes related to schizophrenia in Excel format [12].

2.3 Drugs – Active Ingredients – Diseases – Targets

The potential target of Pollen Typhae and the target protein related to schizophrenia were used in draw

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Venn diagram (http://bioinformatics.psb. Ugent. Be/webtools/Venn/) takes the intersection, which is the potential target of Pollen Typhae on schizophrenia [13].

2.4 Network Construction

The effective active components of Pollen Typhae and the intersection genes and proteins of screened drug targets and disease targets were introduced into Cytoscape 3.7.2(https://cytoscape.org/)Software, construct gene regulation network, and use force oriented algorithm to make the distribution of nodes more reasonable and get better visual effect. Using string online tools (https://string-db.org/)"Multiple proteins" function to construct protein interaction network (PPI), and screen the core genes of PPI according to the number of connecting genes [14,15].

2.5 Go Functional Enrichment

Using R3.5.3 language software package and "stringi" and "colorspace" packages, go enrichment analysis was carried out on the genes of Pollen Typhae regulatory network, and the histogram and Bubble Diagram of enrichment analysis were drawn.

2.6 KEGG Signal Path Analysis

According to the results of go enrichment analysis, the KEGG pathway was analyzed by using R3.5.3 language software package and "Bioconductor" program package, and the main signal pathway of Pollen Typhae in the treatment of schizophrenia was obtained. The hypergeometric distribution design was used for enrichment analysis, and the significance of enrichment analysis was calculated according to the Benjamin Hochberg correction method.

III. RESULT ANALYSIS

3.1 Screening Results of Active Components of Pollen Typhae

Based on comprehensive database search and literature reports, 33 active components of Pollen Typhae were obtained from TCMSP database (Table I). Among them, 8 components meet the oral bioavailability(OB)≥30% and drug likeness(DL)≥1.8, such as kaempferol-3-o-α-L-rhamnosyl(1→2)-β-D-glucoside_ QT(62.87,0.24), isorhamnetin(49.60,0.31), beta sitosterol (36.91,0.75), etc.

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TABLE I. Potential active ingredients of Pollen Typhae

Mol ID	Molecule Name	OB/%	DL
MOL001439	arachidonic acid	45.57	0.20
MOL001040	(2R)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one	42.36	0.21
MOL006115	kaempferol-3-O-α-L-rhamnosyl(1→2)-β-D-glucoside_qt	62.87	0.24
MOL000422	kaempferol	41.88	0.24
MOL000098	quercetin	46.43	0.28
MOL000354	isorhamnetin	49.60	0.31
MOL006111	Testosterone palmitate	34.14	0.71
MOL000358	beta-sitosterol	36.91	0.75

3.2 Potential Targets of Pollen Typhae on Schizophrenia

Based on the 33 compounds obtained, 1264 potential targets were searched. 10243 potential targets for schizophrenia were obtained from Genecards and OMIM databases. 135 drug disease common targets were obtained by intersecting schizophrenia related genes with potential targets of Pollen Typhae by draw Venn diagram (Fig 1).

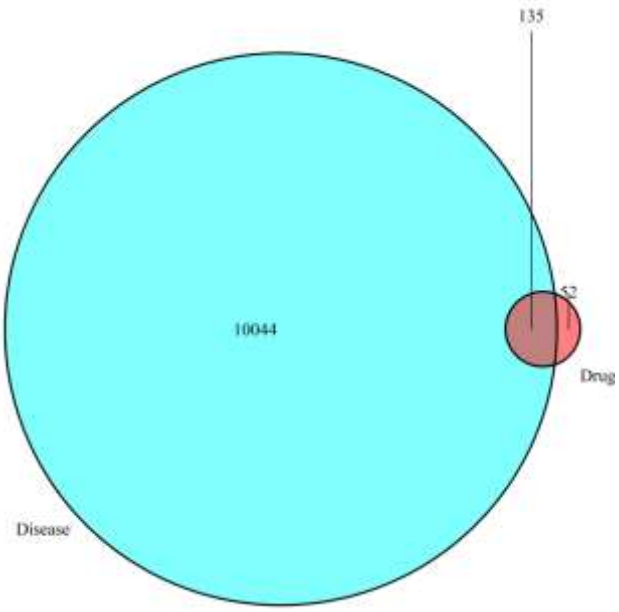

Fig 1: Gene regulation network of Pollen Typhae in the treatment of Schizophrenia

3.3 Network Construction

The network diagram of "active ingredients - drugs - diseases - targets" of Pollen Typhae was constructed by Cytoscape 3.7.2 software (Fig 2). The network diagram contains 142 nodes, 6 active components of Typha and 135 action targets. The most targeted components were kaempferol, luteolin and

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quercetin; the most affected targets were PPARG, ptgs1, PTGS2, ncoa2 and CASP3. Enter the above 135 common targets in the string database and analyze to obtain the PPI network of protein interaction (Fig 3A). Each edge represents the interaction relationship between protein and protein. The more lines represent the greater correlation, and the target ranking in the PPI diagram is obtained. The key proteins are AKT1, IL6 and IL1-β, CASP3, VEGFA, JUN, PTGS2, etc (Fig 3B). These proteins may play a key role in the treatment of Schizophrenia.

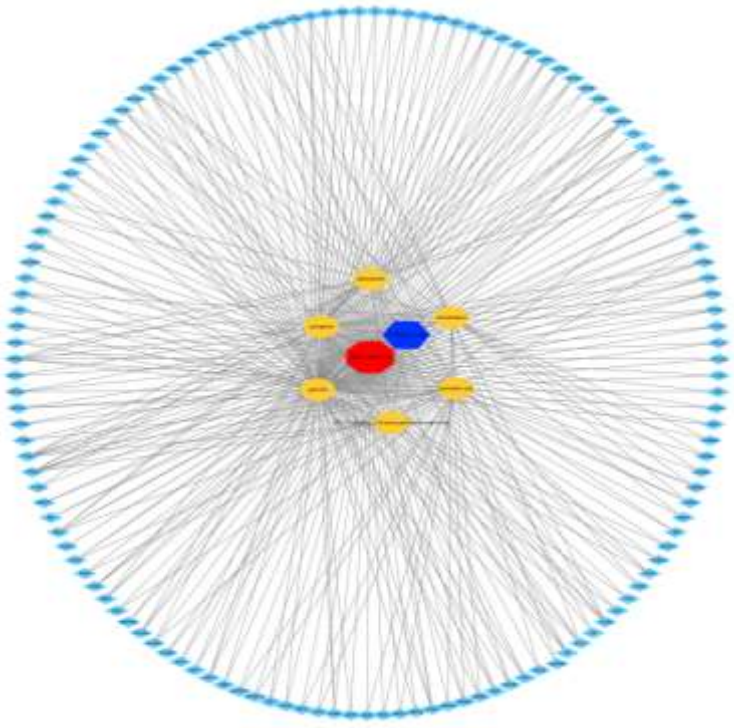

Fig 2: Active component target network of Pollen Typhae

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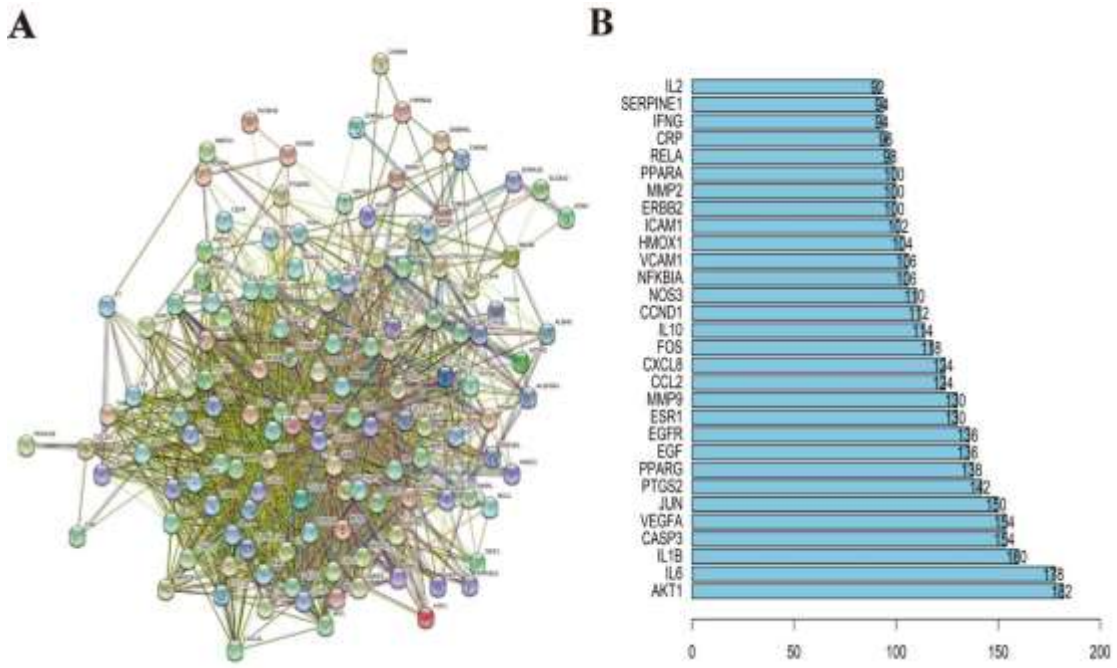

Fig 3: Protein interaction (PPI) network diagram (A) and key protein sequencing (B)

3.4 Go functional Enrichment Analysis

Go enrichment analysis is a directed acyclic diagram composed of the number of genes or proteins at a specific functional level, including molecular function, cell components and biological processes. According to the go analysis of this study, DNA binding transcription factor binding (20/135), RNA polymerase II specific DNA binding transcription factor binding (18/135), amide binding (16/158), receiver ligand activity (16/135) and peptide binding (15/135) ranked top, indicating that relevant targets act on schizophrenia by regulating different biological functions (Fig 4).

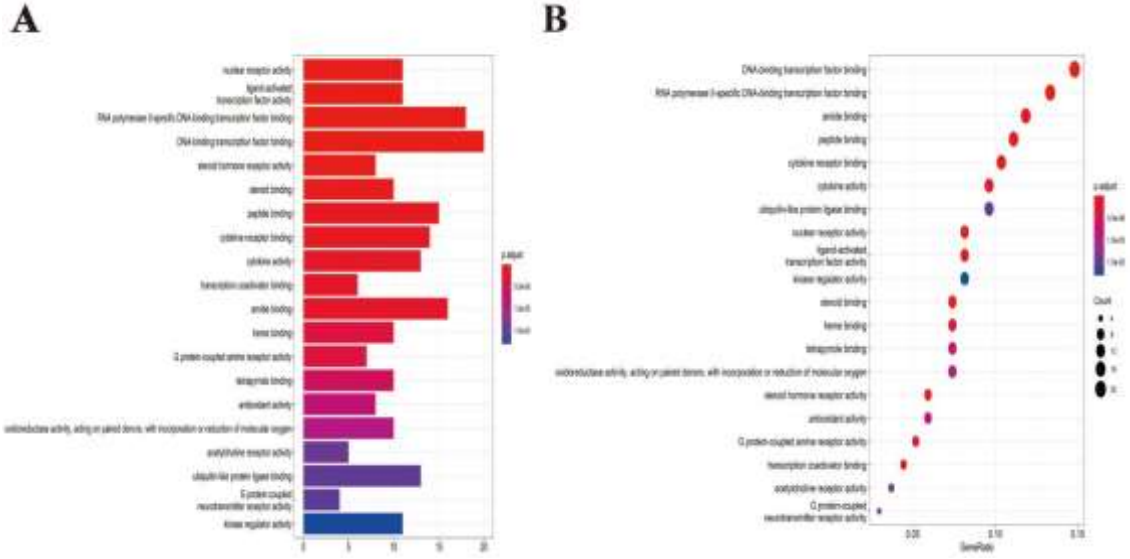

Fig 4: histogram (A) and Bubble Diagram (B) of go functional enrichment analysis

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3.5 KEGG Pathway Analysis

After 135 common targets were run in R language, a total of 166 KEGG pathways were obtained. The results of the first 20 formed a bar graph of KEGG function enrichment. P represents the significance of enrichment, and the redder the color, the higher the significance. The results show that the top signal pathways include lipid and atherosclerosis, fluid shear stress and atherosclerosis, chemical carcinogenesis - receptor activation, AGE-RAGE signaling pathway in Diabetic complexes, Human cytomegalovirus infection, TNF signaling pathway, Chemical carcinogenesis - reactive oxygen species, etc. Among them, the genes involved in Lipid and Atherosclerosis pathway include RELA, CCND1, MAPK1, CASP3, PRKCB, NOS3, MAPK14, BCL2, JUN, PRKCA, AKT1, STAT1, ICAM1, SEL, VCAM1, VEGFA, MMP2, IL-6, F3, IL-1β, CCL2, CXCL8, THBD, Serpine1, IL1A, COL3A1; AGE-RAGE signaling pathway in diabetic complications pathway involves RELA, CCND1, MAPK1, CASP3, PRKCB, NOS3, Mapk14, BCL2, JUN, PRKCA, AKT1, STAT1, ICAM1, SEL, VCAM1, VEGFA, MMP2, IL-6, F3, IL1B, CCL2, CXCL8, THBD, Serpine1, IL(Fig 5).

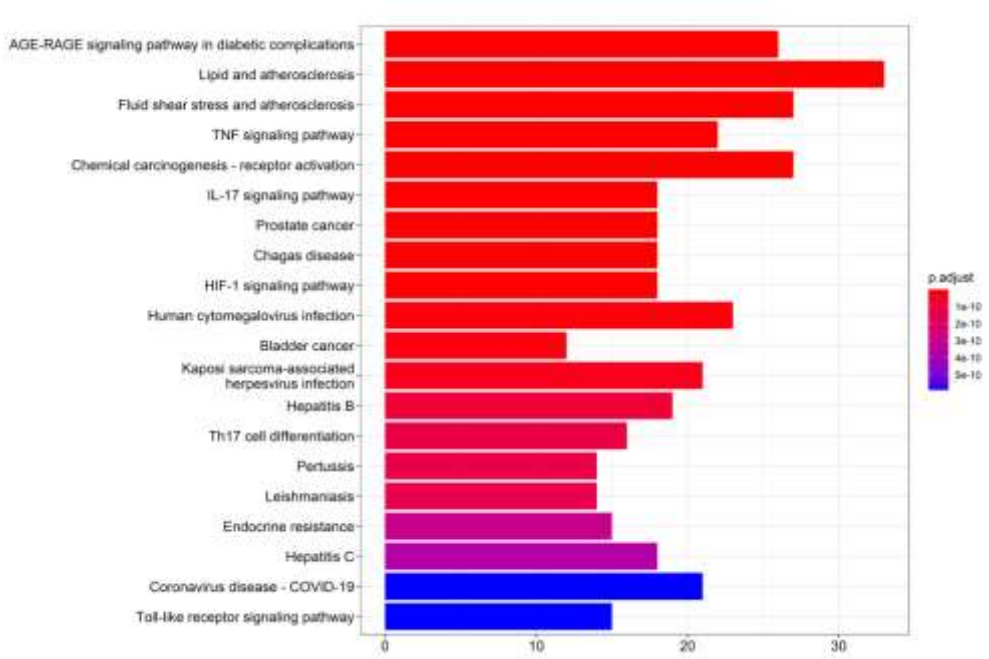

Fig 5: KEGG pathway analysis diagram

IV. DISCUSSION

According to literature reports, the main chemical components of Pollen Typhae are flavonoids, sterols, alkanes, organic acids, polysaccharides and tannins. The components reported in the study are among the 8 effective compounds screened by TCMSP in this study [16]. At the same time, 1624 pharmacodynamic targets of Pollen Typhae were screened, and 10243 schizophrenia related gene targets were screened, including 135 targets of honeysuckle and Alzheimer's disease. According to the screened possible target genes of Pollen Typhae for the treatment of Schizophrenia, the gene regulation network

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map was established by Cytoscape and the PPI map was established by string. The gene regulation network diagram shows the characteristics of Pollen Typhae's multi-component and multi-target treatment of schizophrenia. The PPI results showed that there was a complex interaction between the target proteins of Pollen Typhae. According to the number of connexins, 135 core proteins in PPI, such as PTGS1, ESR1, PTGS2, NR3C2, NR3C1, RXRA and PPARG, were screened. The go and KEGG enrichment pathways were analyzed according to the core proteins.

Many studies have shown that abnormal immune inflammation is closely related to the pathogenesis of schizophrenia. IL-2, IL-6, IL-10 and TNF-α in serum and cerebrospinal fluid of patients with schizophrenia before and after treatment, Many cytokines such as IFN, TNF and CSF and their receptors are abnormal, indicating that the immune function of patients with schizophrenia is abnormal [17]. Studies suggest that the brain can be connected with the peripheral immune system through meningeal lymphatic vessels, indicating that inflammatory factors can enter the blood-brain barrier through blood circulation and the central nervous system through lymphatic circulation [18].

Microglia are a kind of cells with immune function that respond to brain injury and infection in the central nervous system. They can change the transmission ability of synaptic signals by pruning or rearrangeing the connecting framework between nerve cells, and play a role in the inflammatory response of CNS. The microglia in the brain of Schizophrenic patients are more active, and the activity level will gradually increase with the aggravation of symptoms. The inflammatory reaction process of microglia may affect the regeneration, apoptosis and white matter lesions of patients' brain neurons [19]. The signal pathways related to neuroinflammation in microglia include MAPK signal pathway and NF- κB signaling pathway, toll like receptor signaling pathway, peroxisome proliferator activated receptor-γ(PPAR-γ), Notch, NRG1/ErbB4, JAK-STAT and Cholinegic synapse signal pathways. From the results of network analysis, it was found that the effective components of anti-schizophrenia in Pollen Typhae and the signal pathways related to neuroinflammation were MAPK and NF- κB, AGE-RAGE, JAK-STAT, JAK-STAT, NRG1/ErbB4 and Cholinegic synapse signaling pathways all play an important role in the mechanism of nerve cell injury caused by schizophrenia [20]. Therefore, functional prediction suggests that Pollen Typhae may inhibit the activation of microglia, thus inhibiting the excitotoxicity of glutamate, reduce the apoptosis of brain neurons and the nerve damage of oligodendrocytes, and then improve the symptoms of schizophrenia [21,22].

In conclusion, multiple active components in Pollen Typhae can act on more than two targets at the same time, and correspond to multiple signal pathways to play a role in anti-inflammatory, antioxidant and inducing apoptosis. It shows that Pollen Typhae interferes with the molecular mechanism of schizophrenia through multiple channels and multiple targets, and its main functions are to inhibit the abnormal activation of microglia, regulate the metabolism of glutamate, antioxidant. At present, the pathogenesis of schizophrenia is not clear, and the therapeutic mechanism of Pollen Typhae needs to be developed. However, with the help of network pharmacological research, Pollen Typhae can intervene the occurrence and development of schizophrenia from multi-component, multi-target and multi-way, so as to provide data and theoretical support for the functional components and target mechanism of Pollen Typhae in the

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prevention and treatment of schizophrenia.

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REFERENCES

[1] Xingyue Zhang, Jingjing Yao, Yiding Lv, et al. Research Progress on pathogenesis and therapeutic targets of schizophrenia. Journal of International Psychiatry, 2018, 45(02): 201-204.
[2] Mark Weiser, Michael Davidson, Shlomo Noy. Comments on risk for schizophrenia. Schizophrenia Research, 2005, 79(1).
[3] Li Lu, Yiling Shi. Research Status on Pathogenesis and Therapeutic Targets of Schizophrenia. Medical Recapitulate, 2015, 21(09): 1586-1588.
[4] Chinese Pharmacopoeia Commission. Pharmacopoeia of the people's Republic of China: China Pharmaceutical Science and Technology Press, 2010.
[5] Yu Chen, Fengtao Li, Weiwei Tao, et al. Chemical Constituents of Typhae Pollen. Natural Product Research and Development, 2015, 27(09): 1558-1563.
[6] Zenghua Jiao, Yajun Yang, Xiwang Liu, et al. Research Progress on pharmacological action of Typhae Pollen [J]. Journal of Traditional Chinese Veterinary Medicine, 2017, 36(03): 85-88.
[7] Cailian Chen, Taolin Wang, Liucai Zhu. Research Progress on pharmacological action and clinical application of Typhae Pollen. Electronic Journal of Clinical Medical Literature, 2016, 3(08): 1577-1578.
[8] Zhengqi Wang. A traditional Chinese medicine mixture for treating paranoid schizophrenia: CN104399006A. 2015.
[9] Jinlong Ru, Peng Li, Jinan Wang, et al. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. Journal of Cheminformatics, 2014, 6(1).
[10] Junmei Wang, Tingjun Hou. Advances in computationally modeling human oral bioavailability. Advanced drug delivery reviews, 2015, 86.
[11] Qiwan Hu, Feng Mudong, Luhua Lai, Jianfeng Pei. Prediction of Drug-Likeness Using Deep Autoencoder Neural Networks. Frontiers in genetics, 2018, 9.
[12] Yunxia Wang, Song Zhang, Fengcheng Li, et al. Therapeutic target database 2020: enriched resource for facilitating research and early development of targeted therapeutics. Nucleic acids research, 2020, 48(D1).
[13] Bingxiang Shen, Facai Wang, Wei Chang, et al. Study on the mechanism of Coptidis rhizoma in treating gastritis based on network pharmacology. International Journal of Traditional Chinese Medicine, 2020, 42(08): 771-776.
[14] Zhang J, Li B B, Huang M Y, et al. Study on the mechanism of Shenqi Jiangtang granules in the treatment of type 2 diabetes based on network pharmacology. Chinese Journal of Chinese Herbal Medicine, 2020, 51(19): 4873-4883.
[15] Zinsmaier. Cysteine-String Protein's Neuroprotective Rol. Journal of Neurogenetics, 2010, 24(3):

Forest Chemicals Review
www.forestchemicalsreview.com
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[16] Lihong Hu, Shiming Fang, Hong Liu, et al. Research Progress on chemical constituents and pharmacological activities of Typhae Pollen. Journal of Tianjin University of Traditional Chinese Medicine, 2016, 35(02): 136-140.

[17] Liu Haibo, Li Cunbao, Chen Dachun, et al. Advances in immunology of schizophrenia. Journal of International Psychiatry, 2012, 39(02): 89-93.

[18] Louveau Antoine, Smirnov Igor, Keyes Timothy J, et al. Corrigendum: Structural and functional features of central nervous system lymphatic vessels. Nature, 2016, 533(7602).

[19] Binlin Shang. Bioinformatics analysis of schizophrenia and experimental study on pregnancy infection model. Kunming Medical University, 2020.

[20] Yanlin Chen, Cheng Chen, Zeping Li, et al. Research Progress on the relationship between NRG1 / ErbB4 signaling pathway and mental diseases. China Journal of Modern Medicine, 2020, 30(15): 44-49.

[21] Oya Kazuto, Kishi Taro, Iwata Nakao. Efficacy and tolerability of minocycline augmentation therapy in schizophrenia: a systematic review and meta-analysis of randomized controlled trials. Human psychopharmacology, 2014, 29(5):

[22] Zhenzhen Tian, Yiyong Xu, Jinhua Zhu, et al. Study on the intervention of Wendan Decoction on cognitive impairment of schizophrenia based on BDNF/TrkB/CREB signal pathway. Jiangxi Journal of Traditional Chinese Medicine, 2021, 52(04): 33-36.